Cruciferous Indole Derivatives as Dietary Supplements

Confusion between IC3 and DIM

April 2006

The Point Institute
Director: Thomas Guilliams Ph.D.
Website: www.pointinstitute.org
Email: info@pointinstitute.org

Cruciferous Indole Derivatives as Dietary Supplements
Confusion between I3C and DIM

Over the past year we have received many inquires for our opinion concerning various comments about the use of indole-3-carbinol (I3C) and its dimeric condensation product 3,3'-diindolylmethane (DIM) as dietary supplements. Most of these questions stem from a lecture given by Michael Zeligs M.D.[a], along with various other lectures and papers circulating on the internet.[b] In these lectures and papers, Dr. Zeligs states unequivocally that the use of I3C in human subjects is dangerous- even toxic- and likely to result in the increased risk of cancers; while the use of DIM (especially the bioavailable form for which he holds a patent) results in all the potential positive benefits attributed to I3C, without any of the negative potential. If these statements are true, and we contend that they are not, clinicians and their patients would rightly be concerned in using such products.

This short paper will not be a point-by-point rebuttal of each statement which we find misleading or false. Also, we do not propose to know the motivation behind popular statements concerning I3C and DIM; though it concerns us greatly that individuals with financial interest in the outcome of the debate are given such uncritical hearing on these issues.[c] We will attempt to point out the complexity of the current data concerning cruciferous indoles as foods and dietary supplements. Furthermore, we will show how confusion has come when the issues are oversimplified, selectively represented and positioned as if the data points to a scientifically verified conclusion.

We believe the current data suggests that both I3C and DIM have great potential as safe cancer-preventatives in humans. Obviously, more studies need to be performed with both compounds (as with any natural substance) to determine their long-term safety in humans with and without cancer.

Cruciferous Indoles

Many of the health benefits derived from eating cruciferous vegetables (*Brassica*-cabbage, Brussels sprouts, broccoli etc.), especially those which are thought to be cancer-preventing, are thought to be derived from the group of secondary metabolites known as glucosinolates (glucobrassicin, glucoraphanin etc.). When these vegetables are cut, crushed or chewed the actions of the enzyme myrosinase (released from the cells) hydrolyses these glucosinolates into other compounds. For instance, glucobrassicin from broccoli and Brussels sprouts converts readily into several compounds including indole-3-carbinol (I3C) when consumed. I3C can be further converted (chemically or enzymatically) into other indole compounds, including DIM. These compounds have generally been thought to be responsible for the various cellular activities that lead to chemoprevention. [1,2,3]

---

[a] Reversing Estrogen Dominance: Intervention with Cruciferous Diindolylmethane (DIM). at a meeting of the American College for the Advancement of Medicine (ACAM) at their November 2004 meeting in San Diego.

[b] Most notably www.dimfaq.com – a site used to promote the use of Dr. Zeligs' absorption-enhanced formula of DIM

[c] The one notable exception was an article published by LifeExtension Magazine in January 2002. A copy can be found online at www.lef.org/magazine/mag2002/jan2002_report_i3c_01.html (This LE Magazine article is also limited due to broad generalizations and selective representation of the available data)
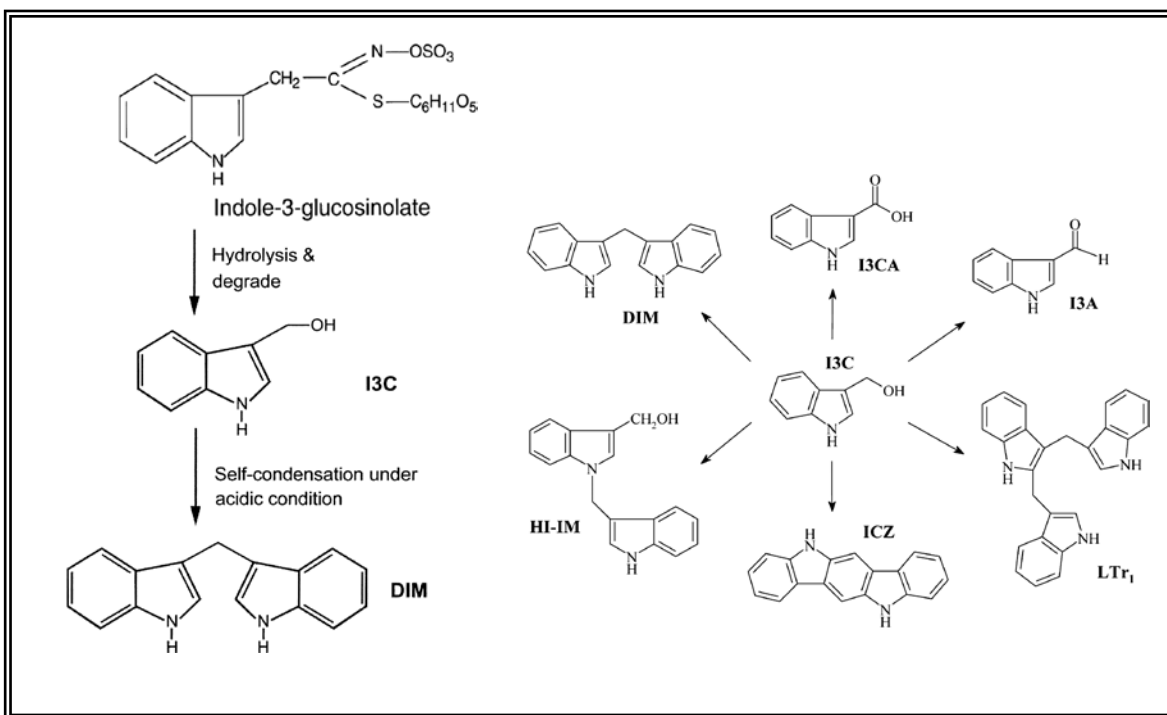
Glucobrassican is converted to I3C- which may be converted to many other compounds.

Complex Data-Confusing Results

       Much of the confusion in understanding the data concerning the chemo-preventative (or other) effects associated with these compounds is due to the diverse types and quality of experiments performed with I3C and its derivatives. Ultimately, what we would like to know is: Can I3C, DIM, or other indole derivatives act as reliable preventative or treating agents for various cancers when given orally to humans? Epidemiological data in populations that consume higher levels of cruciferous vegetables combined with known activities of indole compounds in cell culture experiments have prompted these inquiries.[4,5,6]

       The difficulty exists in the relationship (or lack thereof) between experiments which look at biochemical interactions between molecules, *in vitro* cell culture data (involving different cell types), animal experiments (using different species, variants and induction schemes), and human clinical outcomes. Add to this the unknown pharmacokinetics and pharmacodynamics of these compounds when taken orally by humans, we have only scratched the surface of the issues that need to be addressed in order to better understand the question at hand. These issues are not unique to these compounds, but are quite consistent with many compounds studied across similar fields. This paper is not designed to address all of these issues fully. We will only be able to outline some of the assumptions made about indole compounds and their chemopreventative mechanisms, scrutinize these assumptions with some of the available data and give references for further review. We will mainly focus on those issues and assumptions that attempt to differentiate the benefits or safety between I3C and DIM.

Assumption #1: All the beneficial effects of I3C are due to its conversion to DIM

It was assumed for many years that the effects of oral I3C were due to the gastric conversion into DIM followed by the absorption of this DIM into the plasma and eventually to the target organ(s). I3C was presumed to be too unstable to remain intact, absorb and affect cells within target tissues. This information was based on limited animal and *in vitro* studies. Since that time, many new studies have been published comparing the effects of I3C, DIM and other indoles in various cells; as well as a pharmacokinetic study in animals which challenge these assumptions.

Pharmacokinetics of I3C

The distribution of oral I3C in mice has recently been published showing that I3C absorbs intact and is distributed to many target tissues.[7] In fact, the peak plasma concentration for I3C was 6 and 10 times higher than DIM or LTr (Linear Trimer- see figure), respectively. The I3C concentration was elevated in plasma, liver (highest levels), kidney, lung, heart and brain within 15 minutes of ingestion, but was absent within one hour. DIM, while present in lower amounts, reached its peak 2 hours after I3C dosing and was still detectable in most tissues 6 hours after dosing. Other I3C condensation products were detected within the plasma (I3A, I3CA, HI-IM) although ICZ was only found in liver tissues. These data suggest that oral I3C treatment is likely to result in physiological concentrations of I3C in target tissues.

What happens to I3C when it reaches the target cells? And, more importantly, what differences in cell response occurs when I3C and/or DIM interact with these cells? Those questions are tested by using a surrogate model: Cell culture studies.

Cell Culture Studies

Cell culture studies are extremely helpful in understanding potential mechanisms that various compounds may have within various cells without the complexity of the complete physiology of the animal. This advantage, however, can lead to false conclusions about positive or negative (or toxicity) data generated from these types of studies. In general, I3C, DIM and other indoles have been added to cell culture media and used in numerous cell types (primarily breast cancer, prostate cancer, colon cancer and control cells for each) to find out how gene expression and cellular metabolism is altered by these compounds.

In one study, gene expression profiles were compared in human prostate cancer cells (PC3) treated with either I3C or DIM using cDNA microarray analysis.[8] Gene expression in both I3C and DIM treated PC3 cells were significantly and similarly changed. Genes which were up-regulated tended to be those involved with phase I and phase II detoxification and apoptotic inducers, while those genes down-regulated tended to be involved with signal transduction, oncogenesis, transcription regulation and cell proliferation. Because of the general similarity between the activities of both I3C and DIM with respect to gene regulation (over 700 genes were up or down-regulated by these compounds), and the ability of I3C to convert to DIM, it would be difficult to make many distinctions.

There are many other similar cell culture studies that have compared I3C and DIM gene expression and cellular outcomes. The list below highlights some of the more recent studies to compare these compounds in various cell-culture experiments. There are dozens more articles which one can site here. For reviews on the role of I3C and DIM, as well as proposed mechanisms for cancer prevention, see the following references 16,17,18.

- Both I3C and DIM induce apoptosis in transformed cervical cells. I3C, given orally to HPV16 transgenic mice increased apoptosis and prevented cervical cancer in mice stimulated by estradiol.[9]
- NAG-1 (Non-steroidal anti-inflammatory drug activated gene-1), a gene associated with pro-apoptotic and anti-tumurogenic activities is up-regulated by both I3C and DIM in colorectal cancer cells (HCT-116). In this study I3C and DIM appeared to share some common mechanism, while also having separate mechanisms. The authors also showed synergy between low levels of I3C and resveratrol in the induction of NAG-1 expression.[10]
- I3C and DIM have overlapping, but not identical, activities in G1 cell cycle arrest and induction of apoptosis in breast cancer cells. I3C and DIM may act synergistically within the cell to promote apoptosis. Some conversion of I3C to DIM may even occur within the cell. This review covers much of their work.[11]
- I3C and tamoxifen are synergistic components, arresting the cell cycle in breast cancer cells (MCF-7).[12]
- I3C has a strong anti-proliferative effect on several prostate cancer cell lines and inhibits the production of prostate specific antigen (PSA) from these cells. Synergy was reported between I3C and flutamine, an androgen antagonist that is used as a therapeutic agent for prostate carcinoma. [13]
- Expression of interferon-gamma and sensitivity to interferon-gamma are both increased in breast cancer cells (MCF-7) when treated with I3C.[14]
- Both DIM and I3C act synergistically with genestein in anti-cancer models using MCF-7 cells.[15]

Conclusion for Assumption #1: DIM is an important biological derivative of I3C but does not account for all the biological activities of I3C. Furthermore, I3C acts in much the same manner within cells as DIM; these compounds may even act synergistically.[11] Limited animal pharmacokinetic studies suggest I3C absorbs rapidly and accumulates in many tissues to sufficient levels to account for biological activities. The notion that all, or even most, of the biological effects of oral I3C come from its conversion to DIM alone is not supported by the literature.

Assumption #2: I3C can be harmful, while DIM is always safe.

We have shown above that I3C, as well as DIM have great potential to influence cellular activities. However, if I3C (or one of its non-DIM metabolites) also had harmful

activities when reaching these cells, this could potentially offset its proposed benefits. This has been the contention of many of the DIM proponents. Is there evidence to suggest that this is true, and furthermore, that DIM does not have the same potential harmful consequences?

Before exploring the specific data concerning these indole compounds, it should be noted that many compounds at various doses have been deemed potentially harmful to specific cells or animals. In fact, certain compounds when used in isolation in cell culture or animal studies can have outcomes which are opposite of known mechanisms of protection seen in other models or doses.[19] We have recently seen this in the case of β-carotene, vitamin C, vitamin E, quercetin and other well studied compounds. I3C and DIM are no exception, and these studies must be placed within their proper context before making conclusions on their risk potential in humans.

I3C animal studies

It should first be noted that the studies showing increased carcinogenesis in animals fed I3C were done in animals chemically-induced to tumorogenesis (in many cases, pretreatment of I3C in the same model was preventative!).[d] Also, these experiments (in animals) did not determine whether these negative outcomes were due to the direct activity of absorbed I3C or one of the derivatives of I3C such as DIM. Nonetheless, this information should not be dismissed- but placed in its proper context. The usual studies sited are here.

- Rats given diethylnitrosamine, N-methyl-N-nitrosourea, and dihydroxy-di-N-propyl-nitrosamine had increases in both number and size of glutathione S-transferase placental form (GST-P)-positive liver cell foci assessed at week 24 if they were also fed I3C.[20]
- Rainbow trout exposed to aflatoxin B1 have increased tumor burden if later fed I3C. Both the aflatoxin and I3C showed a dose response dynamic.[21]
- Treating rats with either I3C or beta-naphthoflavone (both blocking agents of DMBA induced tumorogenesis) were unable to suppress carcinogenesis when given 3 weeks after DMBA initiation.[22]

Much has been said about these studies, especially the rainbow trout study, concerning the potential of I3C to harm those taking it by increasing their risk of cancer. Since these initial studies were not repeated with DIM, it was difficult to assess whether these outcomes were due to I3C, DIM, another I3C derivative, or a combination of one or more of these compounds. The premature conclusion by those promoting DIM products was that I3C and its non-DIM metabolites were the culprits and should be avoided. We believe these models are poor surrogates for judging potential human risk, but are valuable in suggesting areas of potential concern when conducting future studies.

DIM is more tumorogenic in Rainbow Trout Liver model.

---

[d] Dr. Zelig's falsely likens these models to the current situation in all humans with exposure to similar environmental carcinogens (ACAM talk). These animal models, however, are not intended to be models of environmental *exposure*, but are elevated doses of specific carcinogens used to *induce* tumors in susceptible animals.

Expanding on their previous studies,[21] researchers at Oregon State University looked at the mechanism behind the tumor promoting activities of I3C, but this time also included DIM in their analysis.[32] In this model, both I3C and DIM acted similarly to estradiol in promoting gene regulation consistent with tumorogenicity, although DIM consistently up-regulated these genes more than I3C.[e] The authors conclude "these data also suggest DIM may have a greater promotional potency than I3C in the trout tumor model based on this mechanism."

Again, while we believe this is strong evidence to suggest that there is little, if any, difference in the expected therapeutic or safety outcomes between I3C and DIM, we believe these models still have extreme limitations in determining risk in humans. We will show below that in some models DIM itself seems to up-regulate genes (CYP19/aromatase, CYP1B1) which could have potential negative outcomes, although we do not conclude that DIM is more harmful than I3C with these preliminary data.

Studies on Ah receptor-mediated outcomes

The expression of Cytochrome P450-1 enzyme genes (CYP1A1, CYP1A2, CYP1B1) are regulated by the aromatic hydrocarbon (Ah) receptor. CYP enzymes play a role in phase I detoxification of both endogenous as well as exogenous compounds (including polycyclic aromatic hydrocarbons). Dioxin binds strongly to the Ah receptor leading to high levels of CYP1A1 expression and activity. Modulation of CYP gene expression and enzyme activation has been one of many mechanisms associated with I3C and its derivatives. These similar mechanisms have led to the concern that I3C or its metabolites could act as a dioxin-like toxin. It should be noted that CYP1A1 is also responsible for increasing 2-hydroxylation of estrogens, thought to be one of the anti-cancer mechanisms of indoles.

In a recent review on the role of Ah receptor mediated toxicity, Nebert et al. caution that "*in the intact animal the role of CYP1 in detoxification versus activation to cause toxicity is likely to depend on the sub-cellular content and location, the amount of Phase II metabolism, the degree of coupling to Phase II enzymes, and the cell type- and tissue-specific context, as well as pharmacokinetics (route and administration, target organ) of the chemical under study. The notion that CYP1A1 is causative in PAH-mediated toxicity and carcinogenesis …may not be warranted and, in fact, the contrary may be true.*"[23] We agree and suggest that these data only show potential mechanisms in isolated cells and conditions and must be used cautiously when extrapolated into potential clinical settings. The papers listed below for I3C, DIM and others must be understood as having the limitations of being cell culture studies.

- CYP1A1 gene expression is similarly up-regulated in prostate cancer cells by both DIM and I3C. DIM up-regulated CYP1B1 (responsible for catalyzing the production of 4-OH estrogens- see below) in these cells while I3C resulted in no change.[8]
- In human breast cancer cells (T47D) both DIM and I3C bind weakly to Ah receptor and both inhibit the activity of dioxin to induce CYP1A1 mRNA.[24]

---

[e] The DIM used in this study was obtained from BioResponse.

- DIM can induce CYP1A1, 1B1 and CYP19 (Aromatase) in H295R cells via multiple pathways, including Ah receptor binding activity.[25]
- ICZ has both antiestrogenic and estrogenic activities in MCF7 cells because of its differential binding to the Ah receptor and estrogen receptor.[26]
- The binding affinity to the Ah receptor is greater for DIM than it is for I3C.[27,28]

Other I3C derived compounds

Up to this point we have covered data primarily reported for I3C, DIM or both. Other compounds can be formed when I3C is ingested and some of these other compounds may have biological activities which are potentially beneficial or detrimental to health. ICZ (indol(3,2-b)carbazole) is often sited because of its higher affinity to the Ah receptor, along with the trimer derivatives: cyclic trimer (CTr), and linear trimer (LTr).

- CTr is a strong agonist of the estrogen receptor which is completely reversed by methylation. Similarities to tamoxifen have been suggested. The biological implications of these findings are yet to be elucidated. [29,30]
- ICZ inhibits gap junctional intercellular communication and bind the Ah receptor in rat primary hepatocytes.[31]

Conclusion- Assumption #2

Selective and premature data have been used to suggest that DIM is "safe", while its parent compound I3C is fraught with potential harm. The models used so far seem to suggest that these compounds cannot be differentiated in this manner. We believe that current evidence suggests that both compounds have great potential for preventing numerous cancers, but need to be researched for potential negative outcomes in animal and human studies (as we would expect with all such compounds). The additional information that suggests that I3C and DIM may act synergistically in some cells suggests that providing DIM alone would prevent this potential benefit, or any benefit gained by I3C directly. Additional human clinical trials (see below) are being conducted on both compounds so we hope to learn more in the near future.

Assumption #3 DIM needs to be specially formulated to have biological effects

Published data concerning the absorption and pharmacokinetics of DIM in humans is limited. As we mentioned previously, studies in rodents suggest that I3C taken orally will result in both I3C absorption and DIM accumulation in various tissues.[7] Whether this DIM is derived from the conversion within the tissues after I3C absorption or represents gastrointestinal conversion and absorption as DIM is not known. These same researchers studied the pharmacokinetics of different preparations of DIM in the same mouse model.[33] Using both crystalline DIM and BioResponse-DIM (a formula containing 25% DIM), they found that the formulated DIM had a greater absorption (34% in plasma and up to 60% in liver). This is most likely due to the increased solubility of the formulated BioResponse DIM.

However; since BioResponse DIM is only 25% DIM, a person needs to take 300 mg of the formula to obtain 75 mg of DIM (typical commercially available dose). In

order to obtain the same benefit (taking into account the difference of absorption-assuming a similar ratio from the mouse study), an individual would need to consume about 112.5 mg of crystalline DIM. Comparing 300 mg of BioResponse DIM to 112.5 mg of crystalline DIM, the patient needs to decide which provides greater value. No other clinical benefit is thought to be derived from formulated DIM.

Conclusion for Assumption #3
        While formulated (BioResponse) DIM has been shown to have increased absorption when compared to crystalline DIM, advertisements that claim it to be "the only absorbable form" are simply false and misleading.[f] No clinical outcomes have been shown to suggest a difference. In fact, crystalline DIM may be a much more economical form of DIM, even when considering the increased dosing to offset the absorption differences.

Assumption #4: Estrogens: 2-OH is Good, 16α-OH is Bad, 4-OH is Ugly
        It is often assumed that the main benefit of cruciferous indoles is their ability to alter the formation of estrogen metabolites, specifically the ratio of 2-OH estrogens to 16α-OH estrogens to promote a less carcinogenic potential. However; promoters of DIM often claim that I3C, but not DIM, can potentially result in increases of 4-OH estrogens, a particularly harmful estrogen metabolite. While we cannot review all of these issues in detail, we will show that these assumptions are far from conclusive and perhaps, do not account for much of the benefits attributed to the cancer prevention potential of these compounds.

Estrogen metabolites and Cytochrome P450 Enzymes[34]
        One of the first issues we must tackle is the relative cancer promoting (or protecting) properties of estrogens and their metabolites. Estrogen (17β-estrodiol (E2), Estrone (E1) and Estriol (E3)) and their many metabolites have different capacities to bind to estrogen receptors with potentially different cellular outcomes. Enzymes within the cytochrome P450 (CYP) family are capable of metabolizing estrogens to produce hydroxylated metabolites which are (potentially) either more or less carcinogenic.[35] These metabolites are differentiated by the location of the hydroxylation (position 2, 4, 16 etc. on the steroid molecule). Generally, the 2-hydoxyestrone (2-OHE1) and the 2-hydroxyestradiol (2-OHE2) are considered to be more protective or "good", while 16α-hydroxyestrone (16α-OHE1) is considered to be pro-carcinogenic or "bad". The 2/16 hydroxyestrone ratio has often been sited as a marker for potential breast cancer risk (higher is considered to be better). These generalizations, along with the characterization of 4-OHE1 as potentially very bad (although difficult to study due to its low concentrations), have characterized much of the early I3C and DIM research. In essence, I3C and DIM were shown to modify the activities of the various families of CYP enzymes to shift cells to produce more 2-OH metabolites, less 16α-OH metabolites or both- resulting in an increased 2/16 ratio and accounting for the potential anti-cancer effects.

---

[f] While some websites still claim BioResponse DIM is "the only absorbable form", most of the current descriptions now say "absorption enhanced." The product is also subtitled "Bioavailable DIM"

Linking a low urinary $2/16\alpha$-OHE1 ratio to an increased breast cancer risk is not yet conclusive. While some epidemiological and case-control studies suggest a link, others have shown no such link exists.[36] In fact, several contradictory reports exist which suggest we still have more to learn. Tissue levels (rather than urine levels) suggest that breast cancer survival (not risk) may actually be increased with higher 16αOHE1 levels.[37] More studies need to be conducted to determine whether this simple urine test can function as a marker for breast cancer (or other cancer) risk and even more, that changing the ratio using compounds like cruciferous indoles can alter these risks. We will discuss the role of I3C and DIM on estrogen metabolites in humans below.

Finally, many DIM-only advocates often say that intake of I3C can result in the increase of 4-OHE1 while DIM will not cause this to occur. These statements are based on data (like the early rainbow trout studies) which were only done with I3C[38] and is not consistent with what some cell-culture data tell us. In fact, in prostate cancer cells DIM (but not I3C) upregulated CYP1B1 gene expression. CYP1B1 is the enzyme responsible for the formation of the 4-OH estrogen metabolites. Again, depending on how one selects the available data, one could suggest that the formation of 4-OHE1 was the result of DIM which was formed after I3C ingestion.

Conclusion for Assumption #4

Both I3C and DIM alter urinary estrogen metabolites in similar ways. It is still premature to suggest that these measurements are adequate to suggest a change in cancer risk potential in humans. The majority of cell-culture data suggests that these compounds have profound and diverse anti-cancer mechanisms; most of which are unrelated to estrogen metabolites. These urine markers may be a good measure of the biological activity of these compounds in a given patient, but may turn out to be poor measures of direct individual risk potential.

Summary: What we know and what does research in humans tell us?

The information that has been published to date suggests that cruciferous indoles are an important class of compounds for human health, especially as it relates to preventing various cancers. Data spanning from epidemiological reports to *in vitro* assays suggest there is great promise in numerous cancers in both men and women. As with many isolated plant compounds, high doses of particular molecules in certain model systems have contradictory results. Such is the case in a few models with both I3C and DIM. By far, the preponderance of evidence shows these compounds to function as agents which prevent cancer cell growth by numerous mechanisms in multiple cell types. For this reason, researchers continue to study these compounds in humans as well as in animal and cell culture models.

More human studies have been performed using I3C than its metabolite DIM, although both are currently being studied in several clinical trials. Previous studies have demonstrated that 300-400 mg of I3C is well tolerated and increases urinary 2/16 OH estrogen ratios in men and women,[38] obese women,[39] as well as a group of women with SLE (Lupus).[40] Furthermore, a placebo-controlled trial in women with cervical intraepithelial neoplasia (CIN), a precancerous lesion of the cervix, showed that women given 200 or 400 mg of I3C orally were much more likely to have complete regression than those given placebo.[41] I3C (200 mg –BID) was also successful in the treatment of

patients with recurrent respiratory papillomatosis (RRP).[42] The National Cancer Institute is currently funding clinical research on the anti-cancer activity (breast) of I3C, completing and publishing the safety and tolerability aspects of their phase I study.[43]

Compared to I3C, studies using oral DIM in humans are few in number and relatively recent. A 2004 pilot study using DIM (108 mg DIM/day as BioResponse DIM) also showed changes in 2-hydroxylation of estrogen urinary metabolites.[44]Also, DIM (60 mg DIM/day as BioResponse DIM) alter 2/16 hydroxyestrogen levels as well as improved symptoms of cyclic mastalgia in healthy pre-menopausal women.[45] Additional clinical trials are underway to test the role of DIM in humans health prevention.

At this time both I3C and DIM show much promise in the area of preventing various cancers in humans, perhaps even acting synergistically at the cellular level. The *in vitro* data suggest that both molecules cause remarkably similar responses in numerous cell types, resulting in similar outcomes. The promising human clinical data with I3C will likely be repeated with DIM, and both will be researched in larger and better studies. Unfortunately, some within the industry are attempting to differentiate these compounds with selective information and oversimplification of cell culture and animal data. This has led to much confusion for both physicians and patients alike. We look forward to future publications that will shed further light on the role of cruciferous indoles as clinically effective agents.

References:

1. Talalay P, Fahey JW. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J Nutr*. 2001; 131(11 Suppl):3027S-33S.
2. McNaughton SA, Marks GC. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. *Br J Nutr*. 2003; 90(3):687-97.
3. Das S, Tyagi AK, Kaur H. Cancer modulation by glucosinolates: A review. *Current Science* 2000; 79(12): 1665-1671
4. Terry P, Wolk A, Persson I, Magnusson C. Brassica vegetables and breast cancer risk. *JAMA*. 2001; 285(23):2975-7.
5. Giovannucci E, Rimm EB et al. A prospective study of cruciferous vegetables and prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2003; 12(12):1403-9.
6. van Poppel G, Verhoeven DT, Verhagen H, Goldbohm RA. Brassica vegetables and cancer prevention. Epidemiology and mechanisms. *Adv Exp Med Biol*. 1999; 472:159-68.
7. Anderton MJ, Manson MM et al. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. *Clin Cancer Res*. 2004; 10(15):5233-41.
8. Li Y, Li X, Sarkar FH. Gene expression profiles of I3C- and DIM-treated PC3 human prostate cancer cells determined by cDNA microarray analysis. *J Nutr*. 2003; 133(4):1011-9.

9. Chen DZ, Qi M, Auborn KJ, Carter TH. Indole-3-carbinol and diindolylmethane induce apoptosis of human cervical cancer cells and in murine HPV16-transgenic preneoplastic cervical epithelium. *J Nutr*. 2001; 131(12):3294-302.

10. Lee SH, Kim JS, Yamaguchi K, Eling TE, Baek SJ. Indole-3-carbinol and 3,3'-diindolylmethane induce expression of NAG-1 in a p53-independent manner. *Biochem Biophys Res Commun*. 2005; 328(1):63-9.

11. Firestone GL, Bjeldanes LF. Indole-3-carbinol and 3-3'-diindolylmethane antiproliferative signaling pathways control cell-cycle gene transcription in human breast cancer cells by regulating promoter-Sp1 transcription factor interactions. *J Nutr*. 2003; 133(7 Suppl):2448S-2455S.

12. Cover CM, Hsieh SJ et al. Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells. *Cancer Res*. 1999; 59(6):1244-51.

13. Zhang J, Hsu B A JC, Kinseth B A MA, Bjeldanes LF, Firestone GL. Indole-3-carbinol induces a G1 cell cycle arrest and inhibits prostate-specific antigen production in human LNCaP prostate carcinoma cells. *Cancer*. 2003; 98(11):2511-20.

14. Chatterji U, Riby JE, Taniguchi T, Bjeldanes EL, Bjeldanes LF, Firestone GL. Indole-3-carbinol stimulates transcription of the interferon gamma receptor 1 gene and augments interferon responsiveness in human breast cancer cells. *Carcinogenesis*. 2004; 25(7):1119-28.

15. Auborn KJ, Fan S, Rosen EM et al. Indole-3-carbinol is a negative regulator of estrogen. *J Nutr*. 2003; 133(7 Suppl):2470S-2475S.

16. Sarkar FH, Li Y. Indole-3-carbinol and prostate cancer. *J Nutr*. 2004; 134(12 Suppl):3493S-3498S.

17. Kim YS, Milner JA. Targets for indole-3-carbinol in cancer prevention. *J Nutr Biochem*. 2005; 16(2):65-73.

18. Brignall MS. Prevention and treatment of cancer with indole-3-carbinol. *Altern Med Rev*. 2001 Dec; 6(6):580-9.

19. Lee BM, Park KK. Beneficial and adverse effects of chemopreventive agents. *Mutat Res*. 2003; 523-524:265-78.

20. Kim DJ, Han BS et al. Enhancement by indole-3-carbinol of liver and thyroid gland neoplastic development in a rat medium-term multiorgan carcinogenesis model. *Carcinogenesis*. 1997; 18(2):377-81.

21. Oganesian A, Hendricks JD et al. Potency of dietary indole-3-carbinol as a promoter of aflatoxin B1-initiated hepatocarcinogenesis: results from a 9000 animal tumor study. *Carcinogenesis*. 1999; 20(3):453-8.

22. Malejka-Giganti D, Niehans GA, Reichert MA, Bliss RL. Post-initiation treatment of rats with indole-3-carbinol or beta-naphthoflavone does not suppress 7, 12-dimethylbenz[a]anthracene-induced mammary gland carcinogenesis. *Cancer Lett*. 2000; 160(2):209-18.

23. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem*. 2004; 279(23):23847-50

24. Chen I, Safe S, Bjeldanes L. Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (Ah) receptor agonists and antagonists in T47D human breast cancer cells. *Biochem Pharmacol*. 1996; 51(8):1069-76.

25. Sanderson JT, Slobbe L, Lansbergen GW, Safe S, van den Berg M. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1, and 19 in H295R human adrenocortical carcinoma cells. *Toxicol Sci*. 2001; 61(1):40-8.

26. Liu H, Wormke M, Safe SH, Bjeldanes LF. Indolo[3,2-b]carbazole: a dietary-derived factor that exhibits both antiestrogenic and estrogenic activity. *J Natl Cancer Inst*. 1994; 86(23):1758-65

27. Jellinck PH, Forkert PG et al. Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation. *Biochem Pharmacol*. 1993; 45(5):1129-36.

28. Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, Bradfield CA. Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci* . 1991; 88(21):9543-7.

29. Riby JE, Feng C et al. The major cyclic trimeric product of indole-3-carbinol is a strong agonist of the estrogen receptor signaling pathway. *Biochemistry*. 2000; 39(5):910-8.

30. Xue L, Schaldach CM, Janosik T, Bergman J, Bjeldanes LF. Effects of analogs of indole-3-carbinol cyclic trimerization product in human breast cancer cells. *Chem Biol Interact*. 2005; 152(2-3):119-29.

31. Herrmann S, Seidelin M, Bisgaard HC, Vang O. Indolo[3,2-b]carbazole inhibits gap junctional intercellular communication in rat primary hepatocytes and acts as a potential tumor promoter. *Carcinogenesis*. 2002; 23(11):1861-8.

32. Tilton SC, Givan SA et al. Toxicogenomic Profiling of the Hepatic Tumor Promoters Indole-3-Carbinol, 17{beta}-estradiol and {beta}-naphthoflavone in Rainbow Trout. *Toxicol Sci*. 2005 Sep 28; Downloaded from journal website ahead of printing.

33. Anderton MJ, Manson MM et al. Physiological modeling of formulated and crystalline 3,3'-diindolylmethane pharmacokinetics following oral administration in mice. *Drug Metab Dispos*. 2004; 32(6):632-8

34. Lord RS, Bongiovanni B, Bralley JA. Estrogen metabolism and the diet-cancer connection: rationale for assessing the ratio of urinary hydroxylated estrogen metabolites. *Altern Med Rev*. 2002; 7(2):112-29.

35. Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT. Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. *Endocrinology*. 2003; 144(8):3382-98.

36. Ursin G, London S et al. Urinary 2-hydroxyestrone/16alpha-hydroxyestrone ratio and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst*. 1999; 91(12):1067-72.

37. Castagnetta LA, Granata OM et al. Tissue content of hydroxyestrogens in relation to survival of breast cancer patients. Clin Cancer Res. 2002; 8(10):3146-55.

38. Michnovicz JJ, Adlercreutz H, Bradlow HL. Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. *J Natl Cancer Inst*. 1997; 89(10):718-23.

39. Michnovicz JJ. Increased estrogen 2-hydroxylation in obese women using oral indole-3-carbinol. *Int J Obes Relat Metab Disord*. 1998; 22(3):227-9.
40. McAlindon TE, Gulin J, Chen T, Klug T, Lahita R, Nuite M. Indole-3-carbinol in women with SLE: effect on estrogen metabolism and disease activity. *Lupus*. 2001; 10(11):779-83.
41. Bell MC, Crowley-Nowick P, Bradlow HL et al. Placebo-controlled trial of indole-3-carbinol in the treatment of CIN. *Gynecol Oncol*. 2000; 78(2):123-9.
42. Rosen CA, Bryson PC. Indole-3-carbinol for recurrent respiratory papillomatosis: long-term results. *J Voice*. 2004; 18(2):248-53.
43. Reed GA, Peterson KS et al. A phase I study of indole-3-carbinol in women: tolerability and effects. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(8):1953-60.
44. Dalessandri KM, Firestone GL et al. Pilot study: effect of 3,3'-diindolylmethane supplements on urinary hormone metabolites in postmenopausal women with a history of early-stage breast cancer. *Nutr Cancer*. 2004; 50(2):161-7.
45. Zeligs MA, Brownstone PK et al. Managing cyclical mastalgia with absorbable diindolylmethane: a randomized, placebo controlled trial. *JANA*. 2005; 8(1):10-20